Using fingerprint HPLC method to develop and control IgG (IVIG) from Cohn (I+II+III) paste production process

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BIA separations
Leaders in Monolith Chromatography

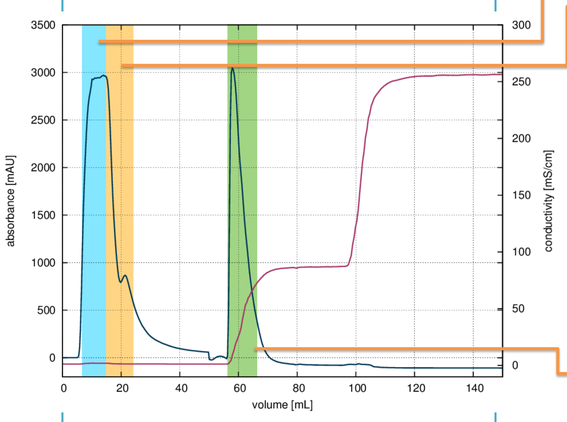
Cohn I + II + III

Dissolution 24 h @ 4°C

Centrifugation 20 min

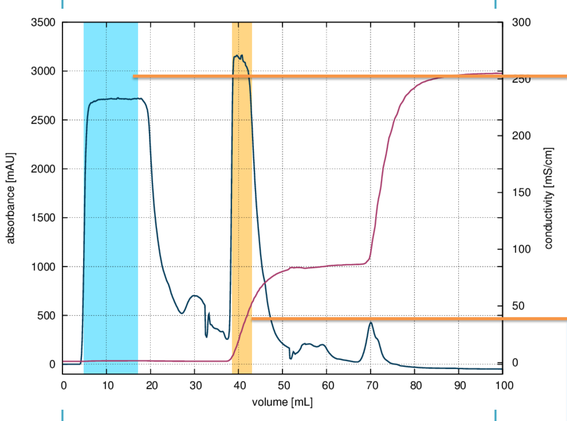
5 % EtOH addition, centrifugation

IEX: CIMmultus QA



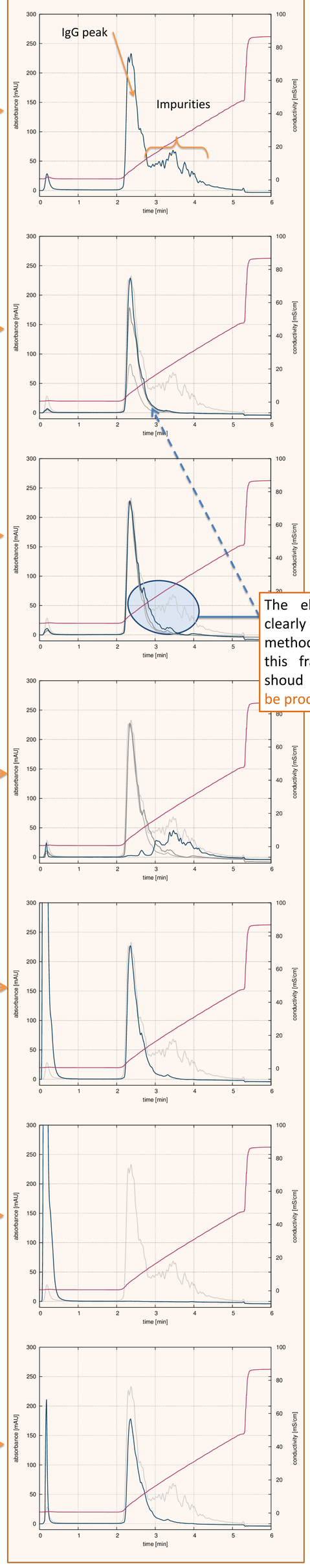
Viral inactivation 16h

IEX: CIMmultus SO3



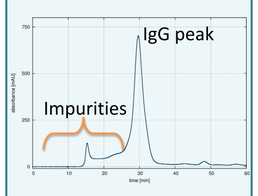
TFF, final formulation

CIMac HPLC fingerprint

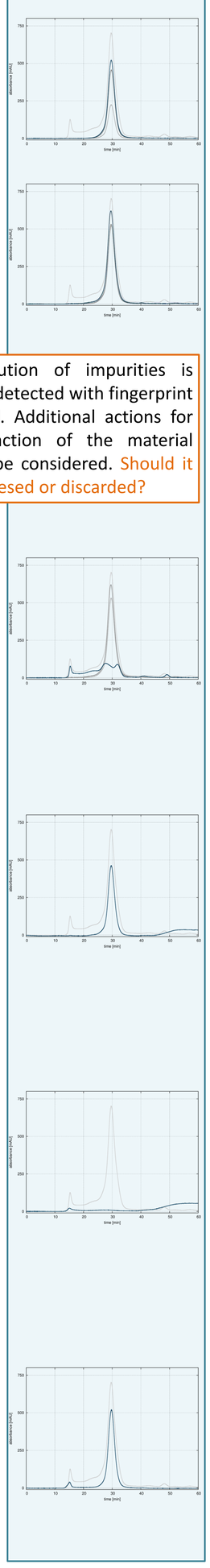


The elution of impurities is clearly detected with fingerprint method. Additional actions for this fraction of the material shoud be considered. Should it be procesed or discarded?

SEC



SEC method
Column: GE Superose 6 10/300 GL
Mobile phase:
50 mM phosphate +
150 mM NaCl, pH 7.2
Isocratic elution
Flow rate: 0.5 mL/min
Load: 50 µL, undiluted sample
Detection: UV at 280 nm



INTRODUCTION

The demand for human immunoglobulin is invariably increasing on an annual basis. To satisfy demands, different manufacturing processes are used to isolate immunoglobulins from human plasma. A quest for alternative paths in manufacturing not only requires development of the most economical manufacturing process, but also a rapid method development and development of reliable analytics for manufacturing monitoring. For an efficient improvement of the purification methods as well as for in-process control during manufacturing stage, the usage of reliable and fast analytical techniques are of crucial importance.

Fast and reliable fingerprint-based method for characterization of immunoglobulin G (IgG) prepared from Cohn I+II+III paste in two chromatographic steps is presented. The fingerprint method bases on partial separation of proteins in linear gradient on CIMac QA 0.1 mL column. Partial separation of proteins does not allow simple quantitative analysis of the samples during the IgG production from Cohn I + II + III paste, however, a very accurate qualitative information about the composition of the sample can be obtained in less than 5 minutes.

FINGERPRINT METHOD

Column:	**CIMac™ QA Analytical Column (CV: 0.1 mL)**	
Mobile phases:	buffer A: 50 mM TRIS-HCl, pH 8.5 buffer B: 50 mM TRIS-HCl, 1 M NaCl, pH 8.5	
Flow rate:	1 mL/min	
Sample loop:	50 µL	
Detection:	UV absorbance at 280 nm	
Gradient method:	Equilibration:	20 CV buffer A
	Load:	15 CV buffer A
	Linear gradient:	30 CV from 100 % buff 50 % buffer A
	Regeneration:	10 CV buffer B
	Re-equilibration:	30 CV buffer A
Sample preparation:	Samples are diluted ten times with buffer A and filtered using 0.22 µm filter before loading onto the column.	

RESULTS

The IgG fingerprint method is based on separation of proteins in linear gradient on CIMac QA 0.1 mL column. At the given conditions IgG elutes in the linear gradient first, followed by the elution of the impurities (with relatively large portions of IgA and IgG). From the differences in the chromatograms of various samples the ratio between IgG and impurities in each sample can be easily assessed.

The method is suitable for input material control, in-line monitoring of the DSP, final control of the products as well as in stability studies. The method allows meeting rapid and accurate decisions enabling thus the production process to be better and more effective.

Preparative columns and conditions

QA step

Column: 8 mL CIMmultus QA
Loading buffer: 20 mM Na-acetate, pH 5.0
Elution: loading buffer + 1 M NaCl
CIP: 1 M NaOH + 2 M NaCl

Sample: 1g Cohn II+III paste in 10 mL loading buffer
Intermediate product: flow through fraction(s)

SO3 step

Column: 8 mL CIMmultus SO3
Loading buffer: 20 mM Na-acetate, pH 5.0
Elution: loading buffer + 1 M NaCl
CIP: 1 M NaOH + 2 M NaCl

Sample: FT of QA step, virus inactivated with Triton-X and TNBP

BENEFITS OF USING PAT

Implementing PAT in the upstream and downstream process brings several advantages and enables to:

- follow the process,
- estimate the robustness of the process,
- optimise the process and cut the costs,
- make quick actions if necessary (during the production),
- controling final product,
- perform stability studies ...

CONCLUSIONS

Due to the high sensitivity, speed and flexibility of the HPLC fingerprint method its implementation into the IgG purification process led to:

- huge time savings, more samples can be analysed in the same time compared to SEC (and SDS-PAGE),
- analysis parallel to the process,
- Possibility to take immediate action,
- quantitative and qualitative analysis of the samples (possibility to be supported by mathematical data processing)

ALL IN ALL MUCH MORE ROBUST IVIG PRODUCTION PROCES RESULTING IN LOWER PRODUCTION COSTS.